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Percutaneous exposure of adjuvant oil causes arthritis in DA rats

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SUMMARY

The arthritogenic properties of adjuvant oil upon percutaneous administration in DA rats was investigated. Groups of rats were administered single or repeated percutaneous applications of Freund's incomplete adjuvant (FIA) or olive oil on shaved skin with or without prior abrasion of the skin. Control rats were shaved and abrased only. A transient arthritis developed in 8/16 animals after repeated applications of FIA on abrased skin. The incidence of arthritis increased to 7/8 animals when FIA was repeatedly administered via filter paper on abrased skin and covered with a bandage. Histological examination of the arthritic joints showed proliferation of the synovial lining layer, infiltration of mononuclear cells and polymorphonuclear cells in the subsynovial tissue. Some bone and cartilage destruction was also seen. Repeated treatment with olive oil on abrased skin induced joint swelling in 3/15 animals, which did not, however, correspond to any microscopically observable signs of inflammation. Also, a single application of FIA on abrased skin or repeated applications of FIA without abrasion induced arthritis, although with low penetration, whereas control animals had no clinical signs of joint involvement. These findings demonstrate that percutaneous administration of adjuvant oil can cause arthritis in genetically susceptible animals.

Keywords adjuvant oil percutaneous exposure arthritis rats

INTRODUCTION

Experimental models of arthritis in rodents, such as adjuvant arthritis (AA) and collagen-induced arthritis (CIA), have been useful in studying the mechanisms of autoimmunity and inflammation involved in the pathogenesis of joint disease. In these, as well as in many other animal models for organ-specific inflammatory disease, the use of immunological adjuvants (agents that non-specifically increase immune responses to specific antigens) is often a prerequisite for the development of disease. However, the exact mode of action of the adjuvant oils in the pathogenesis of these diseases is not fully understood. A new aspect of the *in vivo* effects of adjuvant oils was provided by our finding that adjuvant oil alone can trigger the development of a T cell-dependent and presumably autoimmune arthritis in genetically susceptible animals [1–3]. Thus, mineral oil or synthetic types of oils can induce an erosive polyarthritis (oil-induced arthritis (OIA)) when injected intradermally into DA rats [1]. A question that arises from this finding is whether adjuvant oils can also cause arthritis upon exposures that are common among humans, for example, dermal exposures. Therefore, in this study we have investigated the effects of the percutaneous administration of adjuvant oil with or without simultaneous skin abrasion on the development of arthritis in the DA rat.

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MATERIALS AND METHODS

Animals

DA male and female rats aged 3–5 months were used. The animals were maintained in the animal unit of the Department of Pathology, Uppsala University, and bred from colonies obtained from the Central Institute for Laboratory Animal Breeding, Hannover, Germany.

Oils

Freund's incomplete adjuvant (FIA), composed of mineral oil and an emulsifying agent (Arlacel), was purchased from Difco Laboratories (Detroit, MI), and olive oil was purchased from the University Hospital Pharmacy, Uppsala, Sweden.

Pretreatment of animals

An area of 2×2 cm in the shoulder region of the rat was shaved. Some animals were in addition lightly abrased with sandpaper on the shaved area while under ether anaesthesia. The abrasion was restricted to the epidermis and was repeated twice more during the treatment period at 3–4-day intervals.

Table 1. Incidence of extremity swelling in DA rats following different protocols for percutaneous oil exposure

Treatment	No. with extremity swelling/ total animals	No. with forepaw involvement/ affected animals	No. with hind paw involvement/ affected animals	Microscopic signs of arthritis** in affected animals	Mean day of onset of extremity swelling	Maximum clinical score
FIA*	7/8	6/7	2/7	Yes	15	2
FIA†	8/16	8/8	4/8	Yes	12	3
Olive oil†	3/15	3/3	0/3	No	25	1
FIA‡	1/8	0/1	1/1	ND	30	1
FIA§	1/8	1/1	1/1	ND	23	1
Control¶	0/16					

* Ten applications of 400 μl oil on filter paper and placed on abrased skin under bandage, over a 2-week period.
† Ten applications of 200 μl oil on abrased skin, over a 2-week period.
‡ Ten applications of 200 μl oil without abrasion of the skin, over a 2-week period.
§ One application of 200 μl oil on abrased skin.
¶ Abrased only, over a 2-week period.
** Defined as proliferation of synovial lining layer and subsynovial infiltration of mononuclear cells.
FIA, Freund's incomplete adjuvant; ND, not determined.

Procedures for application of the oils

FIA was applied in a volume of 400 μl or 200 μl directly onto the skin or via a piece of filter paper placed on the shaved area and covered with an occlusive bandage. The adjuvant oil was administered 5 days/week during 2 weeks except for one treatment group that received only one FIA application. Olive oil was applied in a volume of 200 μl directly onto the skin and was administered 5 days/week during 2 weeks. Control animals were shaved and abrased only.

Examination and evaluation of joint inflammation

Animals were examined daily and joints were scored for arthritis onset and severity, using a scale of 1–4 for each paw (maximum possible score of 16 per rat) [1].

Histological examination

FIA- and olive oil-treated animals were killed after various intervals following the first oil application. Ankles and digits were fixed in formalin and decalcified in EDTA. After paraffin embedding and sectioning, conventional haematoxylin and eosin staining was performed.

RESULTS

Development of arthritis upon percutaneous administration of adjuvant oil

Animals with clinically evident arthritis were observed in all FIA-treated groups (Table 1). The highest incidence of arthritis was seen in the group administered repeated FIA applications on filter paper. The initial signs of arthritis were seen 12–15 days after the first FIA application in the groups receiving repeated applications of ajuvant oil on abrased skin. In the group receiving one FIA application only and in the group not abrased, however, there was both a delayed onset and a lower incidence of arthritis.

The arthritis that developed upon percutaneous administration of FIA was in general mild and transient (Fig. 1). The first sign of arthritis was often seen as redness and swelling in a single digit, predominantly in the forepaws. In some animals the arthritis progressed to become symmetric or involve digits or ankles in the hind paws, although forepaw involvement was most common. The highest score of arthritis (with a maximum score of 3) was found in the group abrased and administered repeated FIA applications. The duration of clinically evident arthritis varied, but extended at the most to 9 days (in the group administered repeated FIA applications on filter paper).

Repeated administration of olive oil on abrased skin caused redness and swelling in a single digit of the forepaws of three animals 25 days after the first oil application. No other animals developed arthritis.

Arthritic lesions upon FIA treatment are microscopically characterized by synovitis

Histologically, arthritic joints of the digits or ankles from FIA-treated animals showed proliferation of the synovial lining layer and infiltration of mononuclear cells of the synovia (Fig. 2a,b). An abundance of infiltrating polymorphonuclear cells and mononuclear cells was seen in the subsynovial tissue and in the periarticular tissue. Minimal erosion of bone and cartilage was also observed (Fig. 2c).

Joints from olive oil-treated animals with clinical changes showed vessel dilatations and some proliferation of the synovial lining cells. However, in contrast to the FIA-treated animals, no infiltration of mononuclear cells or polymorphonuclear cells was observed.

DISCUSSION

There has recently been an increasing awareness of the role of adjuvants in the induction of autoimmune reactions and autoimmune diseases. For example, it has been shown that the adjuvant composition determines the development of a given antigen-specific autoimmune disease [4] and that injection of adjuvant oil only can induce arthritis in genetically susceptible individuals (rats with $RT1^{av1}$ [1,5] and mice with H-2q [6]). Such studies further indicate that in these situations the adjuvant itself is the critical agent which triggers otherwise well controlled autoreactive cells to become arthritogenic.

We thus asked in this study whether exposures to adjuvants (mineral oil) that are common in modern human life can cause arthritis in genetically predisposed animals. The types of

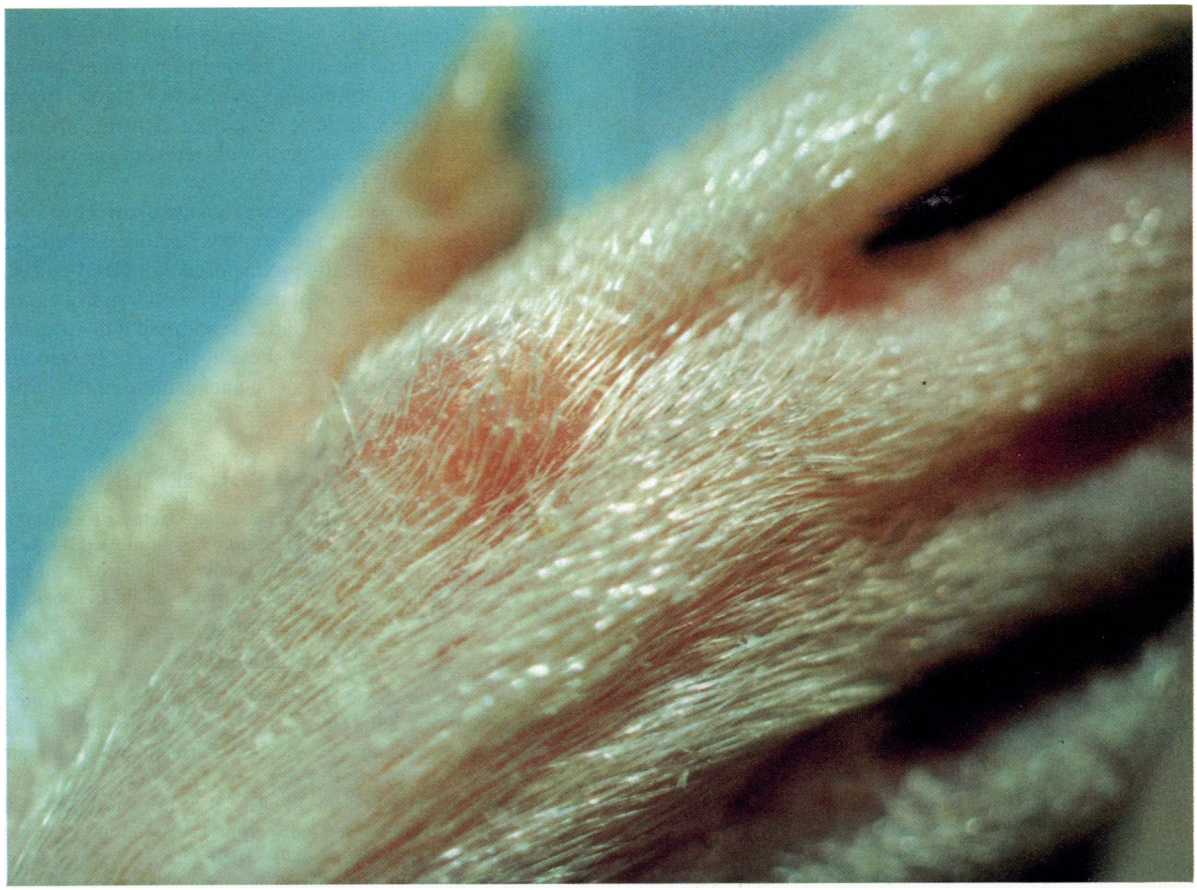

Fig. 1. Hind paw of an arthritic DA rat 20 days after start of treatment with repeated percutaneous applications of Freund's incomplete adjuvant (FIA) via filter paper placed under bandage on abrased skin, showing arthritis in the metatarsal joint.

exposures that were chosen are all of a kind that are commonly encountered in many occupational and other situations. Abrasion represents in this context a way whereby the skin is made more sensitive to immunological stimuli, something that has previously been demonstrated in the development of contact allergy [7].

Our results show that the development of arthritis upon percutaneous administration of FIA was very dependent on the context of the exposure to the mineral oil. Thus, repeated administration of adjuvant oil was more efficient in disease induction than just a single dose; abrasion enhanced the development of arthritis, and this enhancement was most obvious when an occlusion was also added over the adjuvant oil.

The arthritic lesions that developed after percutaneous mineral oil exposure were milder than after intradermal injection of the same oil, and furthermore the localization of arthritis was often confined to the front paws—in contrast to the situation in original OIA, which develops arthritis generally in all paws. This shows that the site and mode of administration of the arthritogenic stimulus may influence not only the severity, but also the localization of the clinical manifestation. Nevertheless, histopathological examination revealed features of the arthritis which were similar to those which have previously been described in the original OIA [1], as well as in adjuvant arthritis [8], collagen-induced arthritis [9] and in many cases of human inflammatory joint disease [10]: infiltration of inflammatory cells, hyperplasia of synoviocytes and erosions of cartilage and bone.

As to the mechanism responsible for the induction of the current arthritis, it appears plausible that the disease is T cell-dependent, as is the OIA induced by mineral oil given intradermally [2,3]. What induces the activation of these putative autoreactive arthritogenic T cells is so far not known. It is conceivable that the mineral oil applied on the skin is primarily taken up by macrophages that are induced to produce certain cytokines, which may in turn contribute to the critical triggering of the disease-inducing T cells. In line with this notion was the observation of the increased arthritis incidence after abrasion of the skin; the local accumulation of inflammatory cells in the skin after abrasion [7] may facilitate both uptake of oil and local T cell activation. The more precise events that are involved in this T cell activation as well as the specificity of the activated T cells are presently subject to further studies in our group.

This investigation has focused on the arthritogenic role of mineral oil, a prototype for an immunological adjuvant. It is plausible, however, that a number of other compounds with adjuvant properties may also have the same effect when applied percutaneously on arthritis-prone individuals; thus even olive oil, which has much less adjuvant activity than mineral oil [11], has the capacity to induce swelling of digits (but not microscopic inflammation) when administered repeatedly on abrased skin.

The present findings invite speculation concerning effects of oily compounds and other chemicals with adjuvant properties in humans. There are obviously a number of situations, for example in occupational life, where there is a heavy percutaneous exposure to mineral oils. Cases of autoimmunity asso-

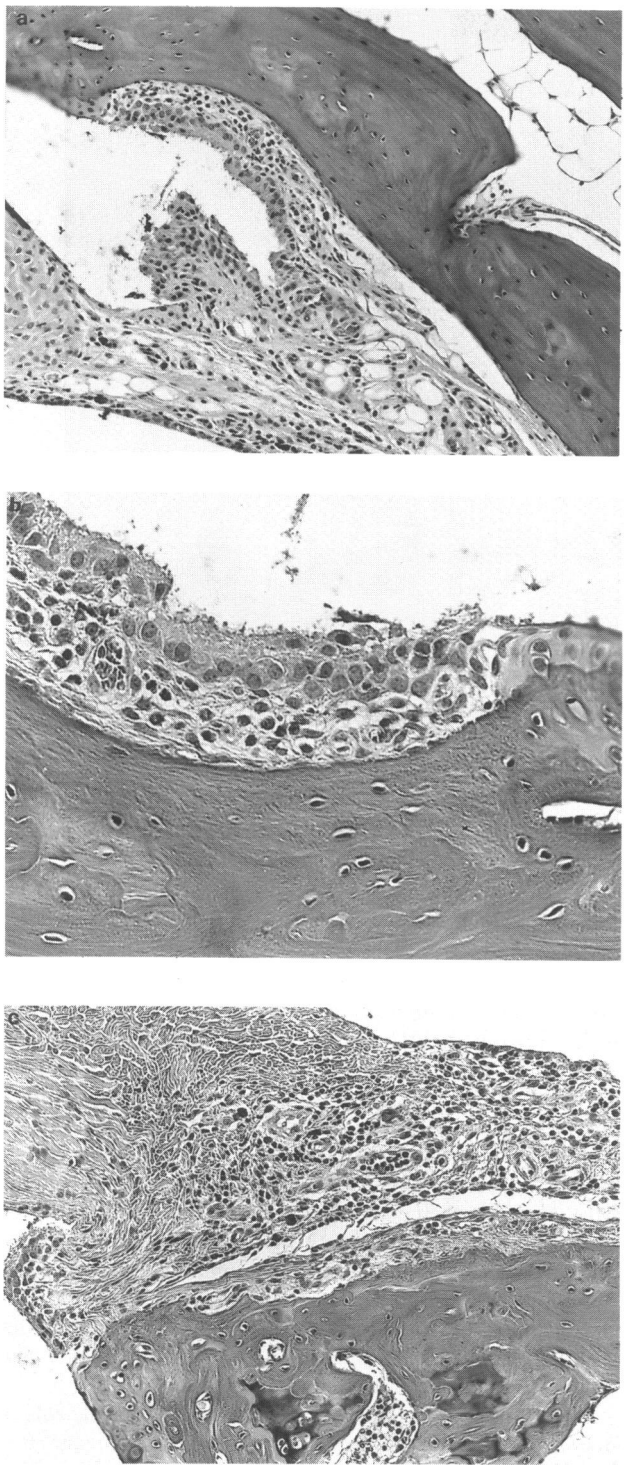

Fig. 2. (a) Section of an arthritic digit 13 days after start of treatment with repeated applications of Freund's incomplete adjuvant (FIA) on abrased skin, showing beginning of pannus formation and an inflammatory villus formation (original magnification ×40). (b) High power magnification of the section shown in a, showing both proliferation of the synovial lining layer and infiltration of mononuclear cells (original magnification × 100). (c) Section of an arthritic digit 13 days after the start of treatment of repeated applications of FIA on abrased skin, showing minimal erosion of bone and cartilage and infiltration of mononuclear cells and some polymorphonuclear cells in the periarticular tissue (original magnification × 50).

ciated with compounds with adjuvant properties in humans have been reported, the most related to our findings being the development of autoimmune connective tissue diseases or autoimmune manifestations (including arthritis, arthralgia) in women after cosmetic surgery with paraffin oil (which is the same as mineral oil) [12,13].

In conclusion, percutaneous exposure to an adjuvant oil can induce an organ-specific, presumably T cell-dependent, autoimmune disease in genetically predisposed animals. In view of this finding there is a need to explore the potential risk of exposure to environmental adjuvants and the development of autoimmunity in humans. Investigations of these agents should be conducted with consideration to the genetic background of the individuals studied.

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